

# Neurotoxic and Pharmacokinetic Responses to Trichloroethylene as a Function of Exposure Scenario

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Strategies are needed for assessing the risks of exposures to airborne toxicants that vary over concentrations and durations. The goal of this project was to describe the relationship between the concentration and duration of exposure to inhaled trichloroethylene (TCE), a representative volatile organic chemical, tissue dose as predicted by a physiologically based pharmacokinetic model, and neurotoxicity. Three measures of neurotoxicity were studied: hearing loss, signal detection behavior, and visual function. The null hypothesis was that exposure scenarios having an equivalent product of concentration and duration would produce equal toxic effects, according to the classic linear form of Haber's Rule ( $C \times t = k$ ), where  $C$  represents the concentration,  $t$ , the time (duration) of exposure, and  $k$ , a constant toxic effect. All experiments used adult male, Long-Evans rats. Acute and repeated exposure to TCE increased hearing thresholds, and acute exposure to TCE impaired signal detection behavior and visual function. Examination of all three measures of neurotoxicity showed that if Haber's Rule were used to predict outcomes across exposure durations, the risk would be overestimated when extrapolating from shorter to longer duration exposures, and underestimated when extrapolating from longer to shorter duration exposures. For the acute effects of TCE on behavior and visual function, the estimated concentration of TCE in blood at the time of testing correlated well with outcomes, whereas cumulative exposure, measured as the area under the blood TCE concentration curve, did not. We conclude that models incorporating dosimetry can account for differing exposure scenarios and will therefore improve risk assessments over models considering only parameters of external exposure. **Key words:** behavior, Haber's Rule, hearing, physiologically based pharmacokinetic model, trichloroethylene, vision. — *Environ Health Perspect* 108(suppl 2):317–322 (2000).

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Risk assessment frequently requires judgment regarding the influence of exposure concentration and duration in producing adverse health effects. Risk assessment decisions often involve estimating safe exposure concentrations for exposure durations that were not tested experimentally. For example, the formulas used in risk assessment to calculate a reference dose (RfD) or reference concentration (RfC) contain uncertainty factors to adjust risk estimates based on data from experiments with less-than-lifetime exposures (1). In addition, exposure levels may also be adjusted to account for differences in exposure duration (2). For example, a non-observable adverse effects level (NOAEL) from a 6 hr/day, 5 days/week inhalation study might be adjusted by factors of 6/24 and 5/7 in an attempt to match the effect of the experimental exposure conditions to those of a relevant environmental exposure (i.e., 24 hr/day, 7 days/week). In addition, the Clean Air Act (3) requires that the U.S. Environmental Protection Agency (U.S. EPA) establish health-based exposure standards governing acute, as well as chronic, exposures; this process entails determining risks associated with exposures over a range of possible durations.

The temporal profiles of actual exposure may vary greatly over time, with relatively

high peaks interspersed among extended periods of lower concentrations. Exposure concentrations that vary over time are often modeled as time-weighted averages. The adjustment of exposure concentration ( $C$ ) by exposure duration ( $t$ ) and the calculation of time-weighted averages relies on the assumption, formulated in Haber's Rule ( $C \times t = k$ ), that the product of exposure concentration and duration produces a constant toxic effect ( $k$ ). This assumption, however, has a poor scientific basis and limited predictive success (4). As an alternative, Andersen et al. (5) advocated adjusting exposure limits over changing exposure durations using physiological pharmacokinetic models and suggested that knowledge about the actions of individual compounds was important in selecting appropriate dosimetry parameters. More recently, characterizing exposure–dose–response relationships has been emphasized (6) as a framework to understand the many factors influencing the relationships between exposure scenario, absorbed dose, target tissue dose, and adverse outcomes.

Volatile organic compounds (VOCs) represent a major fraction of the high-volume compounds released into the atmosphere, and neurotoxicity is a concern following acute exposure to VOCs. For example, 19 of the 25 chemicals with the highest volume of release

into the atmosphere are reported neurotoxicants, and 18 of those are VOCs (7). Trichloroethylene (TCE) was selected as a representative VOC for study because its volume of use, release, and potential exposure is relatively high, and there is extensive pharmacokinetic and neurotoxicity information available (8,9).

The current project represents a coordinated study of neurotoxicity and pharmacokinetics as a function of exposure to TCE over different exposure scenarios, with the ultimate goal of understanding exposure–dose–response relationships. Three specific aims are presented. *a*) The first aim was to determine exposure–effect relationships (i.e., what are the relationships between atmospheric TCE concentration, exposure duration, and neurotoxicity?). To address this, exposure concentration–time relationships were determined for the effects of inhaled TCE on three neurotoxicological outcome measures including hearing loss, signal detection behavior, and visual function. Haber's Rule was proposed as a null hypothesis against which empirically obtained  $C \times t$  relationships were compared. A nonconstant toxic effect was specified as the alternative hypothesis. *b*) The second aim was to establish dosimetric relationships (i.e., how does the concentration of TCE in the blood and brain vary as a function of the exposure?). To accomplish this a physiologically based pharmacokinetic (PBPK) model was developed specifically for inhaled TCE in Long-Evans rats. *c*) The third aim was to determine the

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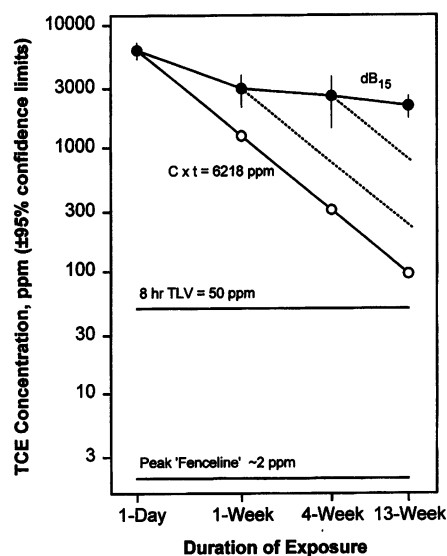
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relationships between tissue concentrations of TCE and functional effects on the nervous system. This aim was accomplished by using the PBPK model to predict tissue TCE concentrations under different exposure scenarios and then comparing the neurotoxic outcomes to estimated tissue dose. This article is organized into three general sections, one for each specific aim. Subsections, where appropriate, cover each individual neurotoxicity outcome investigated. We present only an overview of the results of this research project. The primary reports of the data have been or will be published elsewhere as indicated below.

## The Relationships between the Atmospheric TCE Concentration, Exposure Duration, and Neurotoxicity

### Effects of TCE Inhalation on Hearing Loss

The ototoxicity of a number of VOCs, including TCE, has been observed as a permanent mid-frequency hearing loss in animals and humans (10–14). Extrapolation of ototoxic risk based on this research has been hampered by the short-duration high-concentration exposures used to demonstrate these effects.



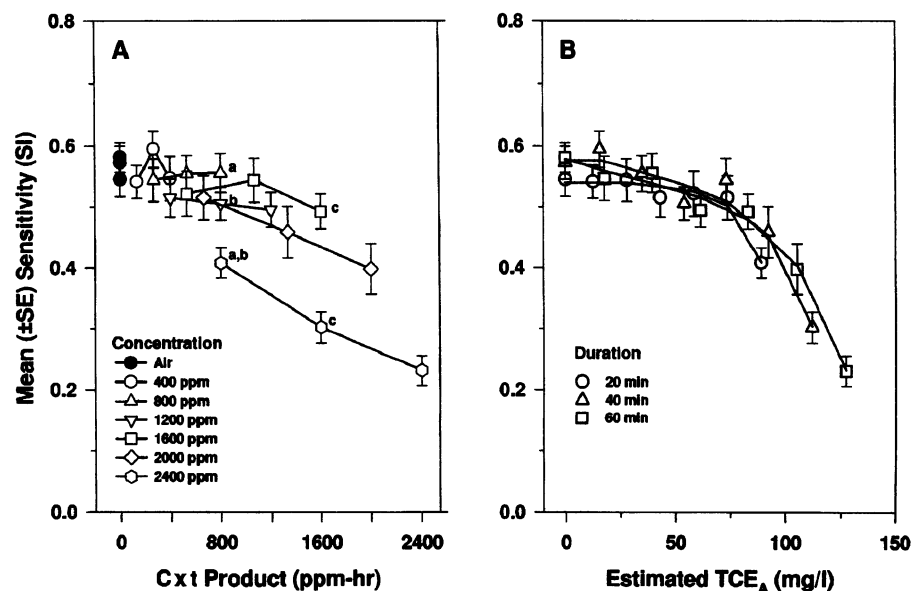
**Figure 1.** A comparison of the theoretical  $C \times t = k$  curve (assuming cumulative effects; open symbols) versus the empirically derived  $dB_{15}$  (filled symbols) for different exposure durations. The  $dB_{15}$  is the concentration at which a 15-dB hearing loss occurs. Vertical error bars represent the upper and lower 95% confidence intervals for the  $dB_{15}$ . The toxicity of TCE is less than that predicted by a strict cumulative effects model. The 8-hr threshold limit value (TLV) and the estimated peak at "fence line" concentration (highest concentration estimated to occur directly downwind from manufacturing facilities during a spill) are shown as horizontal lines. Dotted lines represent  $C \times t$  relationships starting from the 1-week and 4-week exposure reference concentrations. Reproduced from Crofton and Zhao (16).

Thus, we evaluated the adequacy of short-term exposure to high concentrations of TCE for predicting the neurotoxicity produced by exposures to lower concentrations for longer durations. Adult male Long-Evans rats ( $n = 10$ –12 per group) were exposed to air or TCE via inhalation for 6 hr/day for four durations: 1 day (4,000–10,000 ppm); 5 days/week for 1 week (1,000–4,000 ppm); 4 weeks (800–3,200 ppm); or 13 weeks (800–3,200 ppm). These concentrations were based on the initial observation of hearing loss at 2,000 ppm following a 5-day exposure (13). Auditory thresholds were determined for a 16-kHz tone 3–5 weeks after each exposure, using reflex modification audiometry (13,15). This assessment period was chosen due to previous work demonstrating maximum and permanent hearing loss within 3–5 weeks after exposure. Results were modeled using NOAELs, LOAELs (lowest observable adverse effects level), and a polynomial regression that estimated the exposure concentration resulting in a 15-dB sound pressure level increase in the hearing threshold ( $dB_{15}$ ) compared to the control group. A 15-dB elevation of threshold is generally regarded as a clinically significant, adverse effect in humans.

Results replicated previous findings of mid-frequency hearing loss for all exposure durations (13,15). The lowest effective concentrations (LOAELs) for 16-kHz thresholds were 6,000, 3,200, 3,200, and 2,400 ppm for the 1-day, and 1-, 4-, and 13-week

exposures, respectively (Figure 1). The  $dB_{15}$  concentrations were 6,218, 2,992, 2,592, and 2,160 ppm TCE for the 1-day, 1-, 4-, and 13-week exposures, respectively. The  $dB_{15}$  data were fit to a polynomial equation that indicated an extrapolated asymptotic effect of approximately 2,100 ppm for a 2-year exposure (16).

These data suggest that solvent-induced ototoxicity depends almost entirely on concentration at exposure durations lasting several weeks and that the ototoxicity of TCE at these durations was less than that predicted from shorter durations by a strict  $C \times t$  relationship. In other words, use of Haber's Rule to extrapolate from short to long exposure durations underestimated the concentration of TCE necessary to cause hearing loss and thus overestimated the risk of hearing loss associated with exposure to TCE. The fact that hearing loss was not related to the concentration–duration product is probably not caused by the induction of TCE metabolism, since the concentrations involved are well above the level of metabolic saturation. Furthermore, these data demonstrate that the ototoxicity of TCE appears to be restricted to high concentration exposures (i.e., > 2,000 ppm). Because these exposure concentrations greatly exceed most occupational, atmospheric, and residential exposures (8), typical ambient air concentrations of TCE appear to pose a very low risk of ototoxicity to the general population.



**Figure 2.** (A) SI as a function of TCE concentration in air (individual lines) and  $C \times t$  product (abscissa). The three points on each line represent the SI values for the three 0.33-hr (20-min) periods of testing within each 1-hr session. Values are means ( $\pm$  SEM) across rats. Points with matching superscripts at 800 and 1,600 ppm-hr differ from each other ( $\alpha = 0.05$ ). The fact that these points differ indicates that the effect of TCE on signal detection behavior cannot be predicted by the  $C \times t$  product. Reproduced from Bushnell (21). (B) Sensitivity as a function of arterial TCE concentration ( $TCE_A$ ), as estimated by the PBPK model described in the text. Here the data points for each time period during the test session are connected across exposure concentrations. All points and curves are essentially equivalent, indicating that signal detection behavior can be predicted accurately by  $TCE_A$ .

### Effects of TCE Inhalation on Signal Detection Behavior

Concentration–time relationships were examined for the effects of inhaled TCE in rats on performance of a signal detection task in which rats responded to brief, temporally unpredictable visual stimuli that occurred in a fixed location. Accuracy of performance of the task was then used to test the main hypotheses of the project. Adult male Long-Evans rats ( $n = 11$ ) were trained to perform the signal detection task (17) prior to exposure. TCE vapor was drawn into the operant chambers in concentrations of 0, 400, 800, 1,200, 1,600, 2,000, or 2,400 ppm. Exposure durations of 0.33, 0.67, and 1.00 hr were obtained at each TCE vapor concentration, yielding a matrix of  $C \times t$  products. Probabilities of correct and incorrect responses were converted by the theory of signal detection (18,19) to a sensitivity index (SI), which reflects the animal's accuracy in discriminating signals from nonsignal events.

Inhalation of TCE reduced SI; this effect increased with increasing values of both  $C$  and  $t$ , but the effect depended more upon  $C$  than upon  $t$  (Figure 2A). Concentration–effect functions for SI were then generated for each of the three exposure durations used. Criterion concentrations, analogous to benchmark concentrations (20) with continuous data, were computed from each animal's concentration–effect function using an effect level

of a 0.1-unit decrease in SI (10% of full scale). The mean criterion concentration decreased with increasing duration of exposure, confirming the influence of  $t$  in determining the magnitude of the effect. However, the decrease in criterion concentration as exposure duration increased was less than that predicted by Haber's Rule (Figure 3A) (21). Thus, when extrapolating from longer to shorter durations of exposure, the assumption that  $C \times t$  is constant overestimated the concentration of TCE necessary to produce the criterion effect and thus underestimated risk of that exposure.

### Effects of TCE Inhalation on Visual-Evoked Potentials

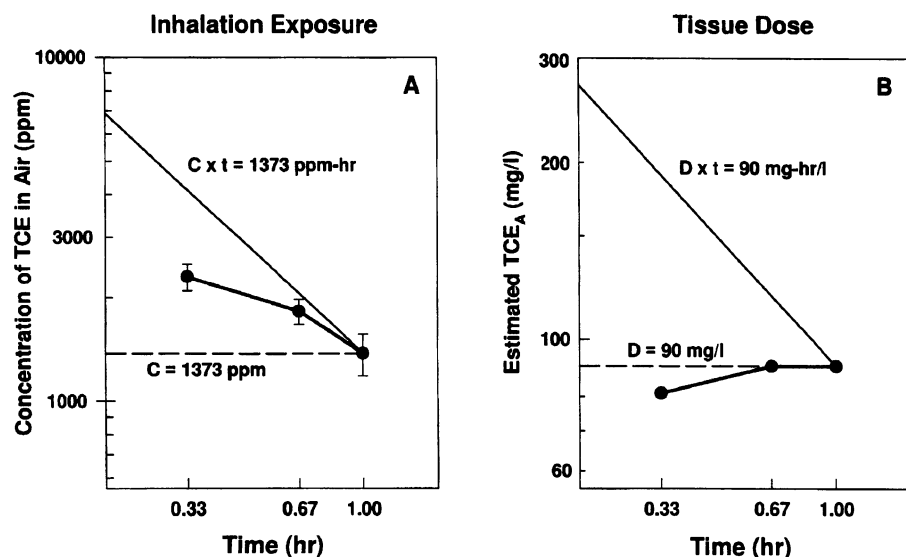
Concentration–duration relationships for inhaled TCE on pattern-elicited visual-evoked potentials (VEPs) were examined to explore the relationship between inhaled TCE and the functional integrity of the visual system. Evoked potentials (22) were recorded from the visual cortex of awake, restrained male Long-Evans rats during inhalation exposure. A pilot study was used to determine an appropriate range of concentration–time combinations to use for testing the null hypothesis, and a value of 4,000 ppm-hr was selected as producing a clear but not maximal change. In a full experiment,  $C \times t$  products of 0 ppm-hr (0 ppm  $\times$  4 hr) or 4,000 ppm-hr were created including 1,000 ppm  $\times$  4 hr; 2,000 ppm  $\times$  2 hr; 3,000 ppm  $\times$  1.3 hr; and

4,000 ppm  $\times$  1 hr ( $n = 9$ –10/condition). If Haber's Rule were a good predictor of outcome, then each group exposed to TCE would show an equivalent effect of treatment.

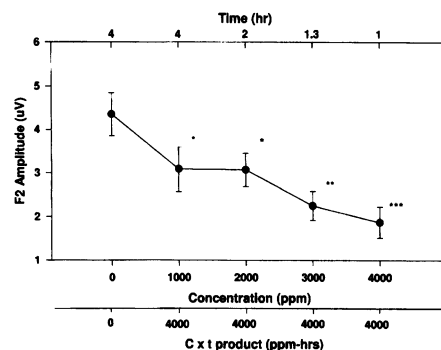
The results showed that VEP amplitude decreased progressively with increasing concentrations of TCE (Figure 4). All groups treated with TCE were significantly different from the air control group, and the groups treated with 1,000 ppm  $\times$  4 hr and 2,000 ppm  $\times$  2 hr were each significantly different from the group treated with 4,000 ppm  $\times$  1 hr. That is, amplitude was reduced as a function of  $C$  but not of  $t$  or the  $C \times t$  product (23). Thus, as was found for hearing loss and signal detection behavior, the prediction of a constant toxic effect for exposure conditions with a constant  $C \times t$  product was not confirmed.

### Summary of Exposure–Effect Relationships for TCE

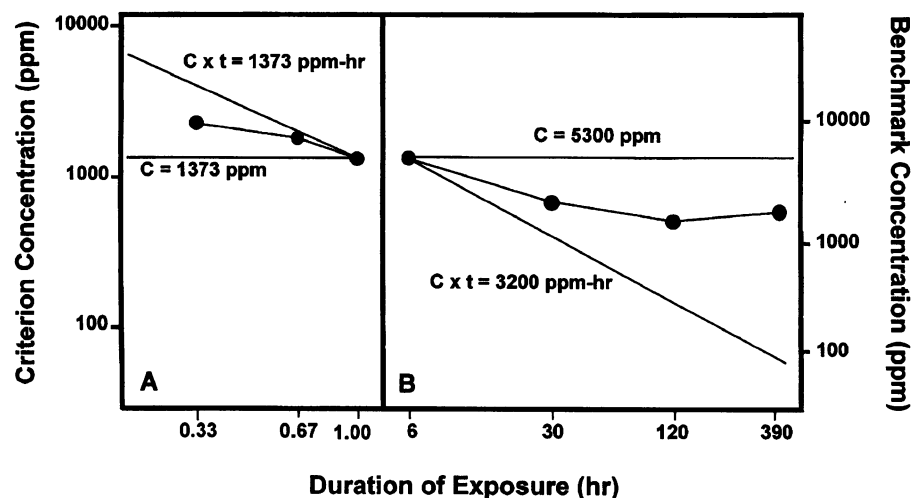
The empirical observations of changes in hearing, signal detection behavior, and visual function show that Haber's Rule does not hold when extrapolating these neurotoxicological outcomes over exposure duration (Figures 1, 2A, 3A, 4). When exposure–effect relationships were generated to determine exposure concentrations producing constant neurotoxicological effects as a result of changing exposure durations, the effective concentrations fell on a line having a shallower slope than that given by Haber's Rule (Figures 1, 3A). Combining these effects schematically (Figure 5) illustrates this relationship: the line representing Haber's Rule is steeper than the lines



**Figure 3.** D, dose (blood TCE concentration). (A) Criterion exposure concentrations of TCE in air (see text) calculated for signal detection behavior (as sensitivity) at each exposure duration, plotted on log-log coordinates as a function of the duration of exposure. The criterion concentrations (points connected by heavy line) increased as exposure time was shortened. The value at 0.33 hr differed significantly from the value at 1.00 hr ( $p < 0.05$ ). Thus, the assumption that time does not influence toxicity (dashed horizontal line) is not supported by the data. However, the criterion concentration did not increase as fast as would be predicted by Haber's Rule—depicted here as the function with a constant  $C \times t$  product (light solid line)—which assumes that  $C$  and  $t$  affect toxicity equally. Reproduced from Bushnell (21). (B) Criterion exposure concentrations of TCE in blood (see text) calculated as in A. Criterion concentrations did not change significantly with decreasing duration of exposure, indicating that time (i.e., the duration for which the tissue concentration is maintained) has little or no effect on signal detection behavior.



**Figure 4.** The amplitude of the frequency double component (F2) of pattern-elicited visual evoked potentials as a function of inhalation exposure concentration, duration, and the  $C \times t$  product. Exposure was to 1,000 ppm for 4 hr, 2,000 ppm for 2 hr, 3,000 ppm for 1.3 hr, or 4,000 ppm for 1 hr, yielding a constant  $C \times t$  product of 4,000 ppm-hr for each exposure condition. A statistically significant reduction in F2 amplitude was produced by each exposure condition. The amount of F2 amplitude reduction was related to exposure concentration, but not to duration or to the  $C \times t$  product. Haber's Law ( $C \times t = k$ ) did not hold. The asterisks designate values that are statistically different from control with a probability of \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .



**Figure 5.** Relationship of functionally neurotoxic concentrations of TCE to changes in the concentration and duration of exposure to inhaled TCE. In both panels, Haber's Rule ( $C \times t = k$ ) forms a straight line with negative slope when plotted on log-log coordinates. (A) Criterion concentrations of TCE (filled symbols) were calculated from observed effects of short-term ( $\leq 1$  hr) acute inhalation of TCE on signal detection behavior. These concentrations fall on a line with shallower slope than the line describing Haber's Rule. (B) Benchmark concentrations of TCE (filled symbols) were calculated from observed effects of long-term (6 hr–30 days) repeated inhalation of TCE on hearing loss. These concentrations also indicate a shallower slope than that predicted by Haber's Rule. The shallower slope indicates that as exposure duration decreases, the concentration of TCE necessary to cause a criterion effect rises. Similarly, as exposure duration increases, the concentration of TCE necessary to cause a criterion effect falls. However, these changes do not occur as fast as predicted by Haber's Rule. Thus, in the short term, Haber's Rule overestimates exposure necessary to cause a criterion effect and underestimates the risk of such exposures. In the long term, Haber's Rule underestimates the exposure necessary to cause a criterion effect and overestimates the risk of such exposures.

representing empirically determined indices of neurotoxicity. Assuming that data are available from an exposure at time  $t_0$  (center point of Figure 5), extrapolation toward longer times using Haber's Rule underestimates concentrations of inhaled TCE associated with hearing loss. Similarly, extrapolation toward shorter times using Haber's Rule overestimates concentrations of inhaled TCE associated with changes in sensitivity in signal detection behavior.

## How Does the Concentration of TCE in Blood and Brain Vary as a Function of the Exposure?

### Pharmacokinetic Model for TCE in Long-Evans Rats

Several PBPK models for TCE are available in the peer-reviewed literature, including models for male F344 rats (24), male Sprague-Dawley rats (25), pregnant F344 rats (26), lactating F344 rats and nursing pups (27), enterohepatic recirculation of TCE metabolites (28), B6C3F<sub>1</sub> mice (29), and humans (30). PBPK models play a prominent role in the U.S. EPA current reassessment of TCE (6,31). Recognizing that various PBPK models for TCE are available, this project developed a PBPK model for TCE in Long-Evans rats for the following reasons: There may be significant strain differences in the pharmacokinetics of TCE (32), and the neurotoxicity data were

collected in Long-Evans rats. Also, none of the existing models account for possible changes in either the distribution or metabolism of TCE that might occur in active, weight-maintained animals, as were used here in the behavioral studies. Further, none of the previously published models include the brain as a defined compartment. The final model is designed to account for the effects, if any, of physical activity and weight maintenance, and to incorporate the brain as a separate, specified compartment.

The initial PBPK model was based on the model structure of Ramsey and Andersen (33), with model inputs either experimentally derived or taken from the literature. Partition coefficients for brain, blood, liver, fat, and muscle were determined in adult male Long-Evans rats fed *ad libitum*, using the vial equilibration method. Tissue/air partition coefficients were calculated using equations derived from Gargas et al. (34) and Sato and Nakajima (35), and tissue/blood partition coefficients were calculated as the ratio of tissue/air to tissue/blood. Marked differences were not observed between the partition coefficients measured in male Long-Evans rats and those measured previously in male F344 rats (24). Tissue volumes for brain, liver and fat were measured in both *ad libitum* and weight-maintained rats (36) so that PBPK models could be constructed for the rats used in the VEP experiments (*ad libitum* food) as well as those used in the behavioral

experiments (restricted food). Blood flow data were those of Delp et al. (37), collected in awake, nonrestrained Sprague-Dawley rats.

A series of uptake curves was generated for TCE in a closed vapor uptake chamber, as has been described for carbon tetrachloride (38,39). Animals were exposed individually so that individual animal variation could be assessed. A PBPK model was used to estimate metabolic constants from the vapor uptake data. Metabolism was assumed to occur only in the liver compartment and was characterized by a single saturable process described by  $V_{max}$  (maximum metabolic rate) and  $K_m$  (Michaelis constant). The metabolic constants were estimated numerically by optimization with Simusolv (Dow Chemical Co., Midland, MI, Version 3.0, 1993).

The PBPK model was used to simulate arterial concentrations of TCE ( $TCE_A$ ) at the time of VEP assessment or during measurement of signal detection behavior.  $TCE_A$  was chosen as the dose metric because of its close relationship to target organ (brain) concentration and because of the ready accessibility of blood from experimental animals. The model predicted tissue concentrations of the parent compound TCE but did not predict tissue levels of metabolites because the expense of analysis of metabolite concentrations made confirmation of model predictions unattainable.  $TCE_A$  was expressed as either area under the curve (AUC) or momentary arterial concentration (i.e., arterial concentration at the time of neurotoxicity assessment). These two indicators of internal dose were then compared to the neurotoxicological outcomes.

## The Relationships between Tissue Concentrations of TCE and Functional Effects on the Nervous System

### Signal Detection Behavior

Values of  $TCE_A$  were estimated by the preliminary PBPK model for each of the  $C \times t$  products tested, both as the average  $TCE_A$  and the AUC for the duration of the 20-min time periods ( $AUC_{20}$ ) during which the behavior was assessed. The magnitude of behavioral change was predicted well by estimated  $TCE_A$  and by  $AUC_{20}$ . That is, the sensitivity of signal detection decreased with relative uniformity as  $TCE_A$  increased (Figure 2B), regardless of the exposure conditions associated with the  $TCE_A$ . In contrast, when the same data were plotted against the  $C \times t$  product of exposure (Figure 2A), clear differences in effect of TCE at a given  $C \times t$  product were evident. These results indicate that estimates of internal dose of TCE predicted functional effects better than did the parameters of external exposure. Furthermore, criterion concentrations calculated for

internal dose of TCE, as  $TCE_A$ , did not change significantly across exposure durations (Figure 3B). This observation suggests that the effect of TCE on signal detection behavior can be described in terms of  $TCE_A$  alone, and that the duration for which this concentration is maintained does not alter the magnitude of the effect.

### Visual Function

The preliminary PBPK model was used to simulate  $TCE_A$  under the exposure conditions previously described for electrophysiological tests. Area under the  $TCE_A$  curve, expressed as the total exposure from the onset to the time of testing at the end of exposure, correlated poorly with VEP amplitude, indicating that the cumulative amount of TCE inhaled was not a critical determinant of VEP changes (Figure 6). In contrast, momentary  $TCE_A$  at the time of VEP recording correlated well with deficits in VEP amplitude.

### Summary of Internal Dose–Effect Relationships for TCE

We observed good correlations between the predicted levels of internal dose and measures of signal detection behavior and visual function. Similar correlations have been observed for avoidance behavior in rats inhaling TCE (40) and after inhalation of toluene (41). In addition, a meta-analysis of the effects of inhaled toluene in rats and humans showed that changes in behavior were systematically related to concentrations of toluene in the blood (42). The measures of internal dose in the present studies included the arterial concentration at the time of testing and the AUC. The latter measure of dose was defined differently for the two outcome measures; for signal detection behavior, AUC was calculated over each 20-min segment of time during which

the behavioral data were collected ( $AUC_{20}$ ) but excluded exposure prior to that time.  $AUC_{20}$  defined in this manner correlated well with behavioral deficits because the behavior was measured throughout this exposure period. For VEP studies, in which VEP assessment took about 1 min, AUC was calculated from the onset of exposure until the completion of testing. In this case, AUC correlated poorly with outcome. For both signal detection behavior and visual function studies, however,  $TCE_A$  at the time of testing correlated well with the magnitude of functional changes observed. It is expected that the concentration of TCE in brain, when measured, will differ from  $TCE_A$  in proportion to the blood/brain partition coefficient and other factors such as the rate of blood flow to the brain. The data to date indicate that  $TCE_A$  at the time of testing predicts changes in neurological function and suggest that the momentary concentration of TCE in the brain may be a critical factor in determining the acute neurotoxic effects of TCE.

It is also of interest to consider whether these effects can be attributed to the parent compound, TCE, or to potentially neuroactive metabolites. The PBPK model indicated that metabolism of TCE was saturated at atmospheric concentrations of TCE above approximately 200 ppm. The fact that there were clear dose–response relationships at concentrations above metabolic saturation, as for example in Figures 2 and 4, suggests that the effects observed were caused by TCE itself and not by any metabolites. These results, however, do not definitely rule out some contribution of TCE metabolites. The contribution of metabolites at the exposure concentrations used here could be assessed by conducting the neurotoxicological experiments under conditions where TCE metabolism is inhibited, assuming that the metabolic inhibitor of choice has no detectable effect on the neurological outcome measure. Alternatively, experiments similar to those described here for TCE could be conducted with various TCE metabolites, such as chloral hydrate or trichloroethanol, to examine the relationship between various measures of metabolite internal dose and neurological effect.

### Research Needs

Further research is needed to characterize the linkage between the pharmacokinetic behavior of the test compound and acute neurotoxic outcomes, and to examine the generality of these conclusions both with regard to other VOC compounds and with regard to human subjects.

The initial PBPK model will be enhanced to include a brain compartment and assessed through comparison of the model predictions with experimental determinations of tissue

TCE concentrations. The PBPK model can then be modified, if necessary, to improve its ability to predict tissue concentrations under the exposure conditions used in the neurotoxicology experiments.

The effects of the prototypic solvent TCE may generalize to those of other solvents, either within the same chemical class (halogenated aliphatic compounds) and/or to other classes (e.g., alkylated benzenes). Future experiments assessing  $C \times t$  relationships associated with exposure to other VOCs are being considered. The degree of generality of these findings will provide guidance for risk assessment strategies regarding the possible mechanisms of action of VOCs in the central nervous system.

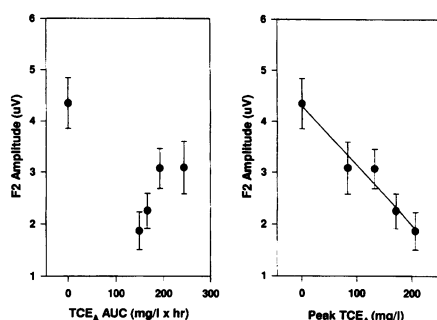
It is also important to assess the generality of these findings in rats to exposure scenarios involving humans. In future studies it should be possible to use equivalent behavioral and electrophysiological procedures in rats and volunteer humans subjects exposed to a common VOC. With appropriate scaling and validation, the PBPK models may predict blood concentrations in both humans and rats. Such direct comparisons may prove valuable for comparing effects of a variety of exposure scenarios in humans and experimental animals (42).

### Conclusions

The research accomplished to date demonstrates that the linear form of Haber's Rule misrepresents the actual risks of exposure when health effects are extrapolated across different exposure concentrations and durations. Consistent conclusions were reached for three measures of neurotoxicity and demonstrate that *a*) when extrapolating from shorter to longer exposure durations, Haber's Rule overestimates exposure risks, and *b*) when extrapolating from longer to shorter exposure durations, Haber's Rule underestimates exposure risks.

Other formulations of Haber's Rule involving power functions of time and/or concentration have achieved better fits to experimental data than Haber's original function (4,21), but these models are still based upon a "black box" approach to the influence of changing exposure scenarios on tissue dosimetry and how those changes influence health outcome (43). When dosimetry is considered, adjustments across exposure scenarios gain a biological foundation.

Ideally, the assessment of risk from a toxic chemical utilizes knowledge of the relationship of exposure to a metric of internal target tissue dose, in addition to demonstrated adverse effects produced in a target organ. Understanding the relationships among exposure concentrations and durations, tissue dosimetry, and adverse outcomes will reduce



**Figure 6.** Evoked potential F2 amplitude for the exposure conditions described in Figure 4, replotted as a function of blood TCE concentration predicted from the PBPK model. The left panel shows F2 relative to  $TCE_A$  expressed as the AUC from the onset of exposure to the time of assessment. The data indicate that AUC was a poor correlate of effect. The right panel shows F2 amplitude as a function of peak  $TCE_A$  at the moment of assessment. Peak  $TCE_A$  correlated well with F2 amplitude reduction.

uncertainties in dose-rate extrapolation, and thus improve risk assessments. In our experiments, when the PBPK model was used to predict blood concentrations achieved under different exposure scenarios, the estimated peak blood concentration at the time of testing accurately predicted the magnitude of effect on visual function and signal detection. The results to date suggest that the acute changes in neural function occurring during exposure are a function of the momentary tissue concentration of the parent compound TCE, although more definitive experiments regarding the influence of metabolism are desirable. Andersen et al. (5) argue that peak blood levels are not generally the most appropriate index of exposure; however, they also point out that the effects of agents interacting reversibly with specific receptors are usually related to peak blood concentration. The acute neuroactive effects of TCE appear to fall in this category.

Regarding assessment of the risk of neurotoxicity from acute exposure to TCE, our results suggest that an appropriate way to extrapolate across exposure concentrations and durations is to determine the tissue concentration produced by exposure at the time of the critical functional assessment, and to use a PBPK model to estimate other exposure conditions yielding the same critical tissue concentrations. The acute effects of organic solvents on the nervous system share many similarities (44,45) but also show unique properties as well (46). Further research will indicate the extent to which these conclusions based on acute exposure to TCE will generalize to the assessment of risk for other VOCs.

## REFERENCES AND NOTES

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